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SPECIFICATION FOR TRYPSIN, MICROBIOLOGICAL GRADE

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Indian Standard

SPECIFICATION FOR TRYPSIN, MICROBIOLOGICAL GRADE

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 30 September 1975, after the draft finalized by the Food Hygiene, Sampling and Analysis Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 Unless the ingredients used in media for microbiological work are of uniform quality, the results obtained might be erroneous and might be unreliable. Since the media used in different laboratories often differ greatly in their quality, the results of microbiological work at different laboratories cannot be compared. Therefore, with a view to unifying the practices of different laboratories dealing with microbiology and providing guidance to the indigenous manufacturers regarding the quality, it was decided to bring out a series of Indian Standard specifications for ingredients commonly used in media for microbiological work.

0.3 Trypsin is a proteolytic enzyme obtained from mammalian pancreas. It is particularly useful in tissue-culture work.

0.4 For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS : 2-1960*. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

1. SCOPE

1.1 This standard prescribes the requirements and the method of test for trypsin, microbiological grade.

2. REQUIREMENTS

2.1 The material shall be in the form of a white to yellowish-white crystalline or amorphous powder.

2.2 It may or may not be completely soluble in water.

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*Rules for rounding off numerical values (*revised*).

2.3 It shall convert not less than 250 times its mass of casein when assayed according to the method given in Appendix A.

2.4 It shall have activity not less than 1 : 250 NF (USA) trypsin units.

3. PACKING, STORAGE AND MARKING

3.1 Packing — The material shall be securely packed in well-filled containers.

3.2 Storage — The material shall be stored in a cool dry place.

3.3 Marking — Each container shall be marked legibly to give the following information:

a) Name of the material including the words ' Microbiological Grade';
b) The units of activity of the preparation, that is, 1 : 250 trypsin unit NF (USA) under conditions of assay for trypsin;
c) Name and address of the manufacturer;
d) Minimum net content;
e) Batch or code number; and
f) Optimum *p*H and temperature.

3.3.1 Each container may also be marked with the ISI Certification Mark.

Note — The use of the ISI Certification Mark is governed by the provisions of the Indian Standards Institution (Certification Marks) Act and the Rules and Regulations made thereunder. The ISI Mark on products covered by an Indian Standard conveys the assurance that they have been produced to comply with the requirements of that standard under a well-defined system of inspection, testing and quality control which is devised and supervised by ISI and operated by the producer. ISI marked products are also continuously checked by ISI for conformity to that standard as a further safeguard. Details of conditions under which a licence for the use of the ISI Certification Mark may be granted to manufacturers or processors, may be obtained from the Indian Standards Institution.

4. SAMPLING

4.1 The representative samples of the material shall be drawn according to the method prescribed in **8** of IS : 6854-1973*.

5. TESTS

5.1 The tests shall be carried out by the method prescribed in **2**.

5.2 Quality of Reagents — Unless specified otherwise, pure chemicals and distilled water (*see* IS : 1070-1960†) shall be employed in the tests.

Note — ' Pure chemicals ' shall mean chemicals that do not contain impurities which affect the experimental results.

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*Method of sampling and test for ingredients used in media for microbiological work.

†Specification for water, distilled quality (*revised*).

APPENDIX A

(*Clause* 2.3)

METHOD FOR THE ASSAY OF TRYPSIN, MICROBIOLOGICAL GRADE

A-1. REAGENTS

A-1.1 Casein (Hammersten) — *See* IS : 7203-1975*.

A-1.2 Sodium Hydroxide — 0·1 N.

A-1.3 Acetic Acid — glacial.

A-1.4 Ethanol — 95 percent.

A-2. SOLUTIONS

A-2.1 Place 0·1 g of fine powdered casein a 50-ml volumetric flask, add 30 ml of distilled water and shake well to bring the casein into suspension. Add exactly 1 ml of 0·1 N sodium hydroxide and heat the mixture at 40°C until the casein is completely dissolved which should not require more than 30 minutes. Cool, add sufficient distilled water to make to 50 ml and mix well.

A-2.2 Dissolve 10 mg of trypsin in 500 ml of distilled water. Mix 1 ml of glacial acetic acid with 9 ml of distilled water and 10 ml of ethanol.

A-3. METHOD

A-3.1 Place 5 ml of casein solution in two test-tubes, and add 2 ml of trypsin solution and 3 ml of distilled water. Mix by gentle agitation. Immediately immerse the test-tubes in a water-bath at 40°C and keep them at this temperature for 1 hour. Remove the test-tubes from the water-bath, add 3 drops of acetic acid mixture to each. There should be no precipitate. The absence of a precipitate indicates that trypsin hydrolyzes not less than 250 of its mass of casein.

NOTE 1 — When a given mass of trypsin hydrolyzes not less than 250 times its mass of casein under the conditions specified above, it is said to have an activity of not less than 1 NF (USA) unit. Such a grade of trypsin is known as 1 : 250.

NOTE 2 — By suitably decreasing the amount of trypsin added to the reaction mixture it may be determined whether it possesses 2 NF (USA) (1 : 500) or more units of activity.

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*Specification for casein hydrolysate (acid digested), microbiological grade.

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